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(54) IMPROVEMENTS IN AND RELATING TO THE PROTECTION OF EDIBLE OR POTABLE PRODUCTS AGAINST ATTACK BY MICROORGANISMS

(71) I, IVAR HARRY SANICK, a citizen of Sweden, of Uppåkra, Lund, Sweden, do hereby declare the invention, for which I pray that a patent may be granted to me, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to a process and a composition for the protection of edible or potable products (hereinafter referred to as "foodstuffs") that are susceptible to attack by micro-organisms such as fungi (in particular mould fungi), and putrefactive bacteria. Such susceptible foodstuffs include meat or fish products or meat- or fish-containing products in a raw, semi-processed or processed state, e.g. meat pies, patés or pastes, such as liver paste and ready-to-eat meat dishes, dough products in a semi-prepared or fully-prepared state, such as wheat or rye bread products, and fruits of different kinds, such as tomatoes and citrus fruit.

It has been known for a long time that many different spices possess an inhibitory or lethal effect on a large number of strains of bacteria and/or fungi. However, a number of difficulties have been encountered when attempts have been made to use such spices for the preservation of foodstuffs. One of the difficulties has been that the spice, in order to be effective, has to be employed in quantities that provide an unintentional and perhaps undesirable flavouring effect.

Although the literature contains rather contradictory information about the anti-bacterial and/or anti-mycotic effect of various flavouring agents—and, at times, even completely incorrect information—it is agreed that cinnamic aldehyde (which is to be found *inter alia* in cinnamon oils) has a significant inhibitory effect on a number of different fungi as well as on various strains of bacteria (cf. F. M. Ramadan et al, "On the Anti-Bacterial Effects of some Essential Oils" in Chem. Mikro-biol. Technol. Lebensm.", volume 1 (1972), page 96 and Clifton F. Lord Jr. and W. J. Husa "Antimolding Agents for Syrups" in J. Am. Pharm. Ass., Volume XLIII, No. 7 (1954), pages 438—440). The corresponding cinnamic acid and its salts are likewise stated to possess an anti-bacterial effect and their use has been suggested for the preservation of particularly fruit and vegetables, cf. U.S. Patent Specifications Nos. 2,790,717; 2,819,972 and 2,819,973). However cinnamic acid and salts thereof have a significantly weaker effect than cinnamic aldehyde.

In practice, however, the use of products containing cinnamic aldehyde as preserving agents is greatly hampered by the fact that the preservative effect often diminishes rapidly, not only when the product is in storage, but also subsequent to having been added to the material intended to be preserved.

The present invention seeks to overcome this problem and to provide an effective and practicable process for the protection of foodstuffs using cinnamic aldehyde as an anti-microbial agent without this resulting (when the anti-microbial composition is added directly to the foodstuffs) in any undesirable flavouring of the foodstuff. The present invention also seeks to provide a cinnamic-aldehyde-containing composition for the protection of foodstuff-products against an attack by micro-organisms, which composition possesses improved storage stability.

Accordingly, the invention provides a process for the protection of foodstuffs (as hereinbefore defined) liable to attack by micro-organisms against the attack of such micro-organisms, in which the foodstuffs, or a source of infection of the foodstuffs by micro-organisms, is treated with an effective amount of cinnamic aldehyde and at least one organic sulphur compound selected from the group consisting of diallyl sulphide, diallyl disulphide, allyl-propyl sulphide, allicin and alliin.

The sources of infection to be treated by the process of the invention may include walls in rooms wherein the foodstuff is to be treated or stored, the people handling the foodstuff, working tools and machines and packing material for the foodstuffs.

The invention also provides a composition for use in the process of the invention, the

composition comprising cinnamic aldehyde mixed with at least one organic sulphur compound selected from the group consisting of diallyl sulphide, diallyl disulphide, allyl-propyl sulphide, allicin and alliin.

It has been found that the period of time during which the cinnamic aldehyde remains effective against micro-organisms, especially fungi, using the process of the invention is extended relative to when cinnamic aldehyde or conventional cinnamic - aldehyde - containing products are employed alone. This is surprising, for although it is known (*inter alia* from H. Dold & A. Knapp "Über die antibakterielle Wirkung von Gewürzen" in the "Zeitschrift für Hygiene und Infektionskrankheiten", *128* (1948) pages 696—706) that the essential oils contained in the bulbs of plants of the Liliaceae family, particularly garlic (*Allium sativum*), possess a good anti-bacterial effect with respect to a number of strains of bacteria, they are not known to possess any anti-mycotic effect of significance.

Cinnamic aldehyde in the process and composition of the invention may be the pure compound or be in the form of a cinnamic-aldehyde-containing product, preferably a cinnamon oil. Commercially-available cinnamon oils, such as cassia oil (normally containing 75—90% by weight of cinnamic aldehyde) and Ceylonese cinnamon oil (normally containing 55—70% by weight of cinnamic aldehyde) may be employed or cinnamic - aldehyde - containing solutions, emulsions or suspensions in solvents which are inert with respect to the cinnamic aldehyde and the organic sulphur compound. The preferred solvent is ethanol, though other solvents such as water may also be employed.

The sulphur compounds used in the process and composition of the invention are characteristic constituents of the essential oils from edible bulbs of plants of the genus Allium of the Liliaceae-family, especially onion (*Allium cepa*) and garlic (*Allium sativum*), and may be used in the form of such oils, garlic oil being the especially preferred oil. Such oils can be recovered from the bulbs in conventional manner by pressing, distillation or extraction, followed, if so desired, by concentrating, fractionating or/and diluting the so obtained "juice", "oil" or "extract". The sulphur compound(s) may also be used in the form of an artificial onion or/and garlic essence, preferably a garlic essence. Most of the commercially-available artificial onion or garlic flavours (or essences) are solutions of diallyl sulphide or diallyl disulphide or allyl-propyl sulphide or mixtures thereof in an inert solvent, normally an alcohol such as propylene glycol or preferably ethyl alcohol. Such artificial onion and garlic essences or flavours may also contain the sulphur-compound allicin

$(C_3H_5—S(O)—SC_3H_5$

soluble in ethanol) and/or alliin

$(C_3H_5—S(O)—CH_2—CH(NH_2)—COOH$

soluble in water, insoluble in ethanol), possibly mixed with diallyl sulphide and/or diallyl disulphide, and/or allyl-propyl sulphide. The sulphur compound may be used in the form of a solution, emulsion or suspension in an inert solvent such as water. The solvent should naturally be one which is suitable for addition to foodstuffs.

As explained above, the cinnamic aldehyde remains effective for a longer period of time than has previously been the case. It is not known exactly why this effect occurs, but it is known that one commercially available cinnamon oil (Ceylonese cinnamon oil) contains fairly large amounts of eugenol which itself possesses a bactericidal effect. It is stated in the literature that the eugenol present stabilizes the cinnamic aldehyde against air oxidation to cinnamic acid. The tests carried out in connection with the present invention show, however, that the anti-mycotic effect obtained with a cinnamon oil having a high eugenol content is not maintained significantly longer than that obtained with other varieties of cinnamon oil having a lower eugenol content. The stabilization achieved with the sulphur compounds by the present invention thus cannot be compared with stabilization with the effect of eugenol. Moreover, the effect achieved by employing garlic oil with cinnamon oil having a high eugenol content appears to be somewhat inferior to that obtained using garlic oil in conjunction with other varieties of cinnamon oil. This may possibly be explained by condensation of cinnamic aldehyde and eugenol. However, the inventor has observed that if eugenol (10% by weight) is added to cinnamic aldehyde, the mixture gradually darkens when stored in an open colourless glass container. Such darkening does not occur if garlic juice is added instead of eugenol under the same conditions. Synthetically produced garlic essence (containing a mixture of diallyl sulphide and diallyl disulphide) possessing a corresponding strength (determined by the ultra-violet photospectrographic method) has the same effect, as does cinnamon oil (approx. 85% cinnamic aldehyde).

In addition, laboratory tests conducted on ripe tomatoes stored at a temperature of +20°C showed that, when treating these tomatoes with a 0.2% v/v solution in ethyl alcohol of the aforementioned mixture of cinnamon oil (or cinnamic aldehyde) and engenol, putrefaction of the tomatoes took place by fungi growing on them in the course of approximately 9 days. Upon replacing the eugenol with garlic extract (or synthetic garlic

essence) the keeping time of the treated tomatoes was increased to approximately 16 days. It was not possible to achieve this effect by treating the tomatoes with cinnamic aldehyde alone or with garlic extract alone in concentrations corresponding to the total concentration of cinnamic aldehyde and garlic sulphur compounds in the mixture.

The combined application of cinnamic aldehyde and sulphur compounds is effective against the ordinary attacks by fungi and putrefactive bacteria on foodstuffs, that is to say, attacks primarily by fungi and bacteria belonging to the genera Penicillium, Aspergillus, Mucor, Candida, Lactobacillus, Saccharomyces, Pseudomonas, Streptococcus, Rhizopus, Salomonella, Achromobacter, Escherichia, Bacillus, Proteus and Clostridium.

The cinnamic aldehyde and sulphur compounds can be applied separately to the material or object that is to be treated, or they can be applied in the form of a mixture. So far as is known, no critical upper concentration exists for the active substance and the lowest useful concentration is determined simply by that required to achieve a satisfactory protective effect, without adding an undesirable amount of ballast material (chiefly the solvent or emulsion liquid). When the compositions of the invention are added directly to foodstuffs, only exceedingly small quantities of active substances are required and to facilitate uniform distribution of the active substances within the foodstuffs and intimate contact of the active substances therewith, it may be advantageous to use dilute solutions and/or emulsions or suspensions of the active substances. In practice the cinnamic aldehyde may conveniently be employed in solution, preferably in ethyl alcohol solution, or in emulsion in concentrations of from 0.001 to 1% by weight, preferably from 0.005—0.5% by weight. The sulphur compounds may conveniently be used in solution, preferably in ethyl alcohol solution, and/or in emulsion in concentrations above 0.5 ppm, preferably from 1 ppm up to 100 ppm, calculated as diallyl sulphide or diallyl disulphide.

The quantity of active substances to be added directly to a foodstuff varies according to the type of foodstuff and the conditions to which it will be exposed prior to consumption. It is possible to determine an effective amount in each individual case by simple empirical tests, but a useful practical rule is to add as much as is possible without producing any undesirable flavouring effect. In most cases, quantities of between 0.001 and 0.1%, preferably between 0.005 and 0.05% by weight of the foodstuff of cinnamic aldehyde and between 0.0001 and 0.01% by weight of the sulphur compounds (calculated as diallyl sulphide or disulphide) will be satisfactory. The ratio between the active substances can vary within fairly wide limits. The preferred ratio of cinnamic aldehyde to sulphur compounds will in most cases be between 20:1 and 100:1. In some cases, for example when preserving fruit such as tomatoes, it is only necessary to surface treat the foodstuff by spraying, brushing or immersion. In these cases, the undesirable flavouring effect will normally be of secondary importance so that the active substances can, if desired, be used in higher concentrations. Such surface treatment may also be used in other cases, for instance, when preserving meat pastes such as liver pastes and other ready-to-eat or semi-processed meat dishes and wrapped sliced bread. In many cases it is useful both to mix the cinnamic aldehyde and the sulphur compound into the foodstuff and to carry out a final surface treatment, as, for example, when producing finished meat dishes such as meat pies or pastes.

In treating sources of micro-organism-infection, a liquid protective composition may be applied by spraying, brushing or otherwise. In these cases the undesirable flavouring effect plays a lesser role, but an upper limit for the quantities used should be set so as to avoid an undesirably strong odour in, for example, the working room.

A previously prepared liquid mixture of cinnamic aldehyde and liquid sulphur product may conveniently contain the active substances in the aforestaid ratio suitable for application, the active compounds being present in a concentrated or diluted state, so as either to be diluted prior to use or in a ready-to-use form. Although ethyl alcohol is the preferred solvent other alcohols such as propylene alcohol or glycerol may also be employed. It is preferred in practice to prepare the mixture by mixing cinnamic aldehyde or commercially available cinnamon oil with onion or (preferably) garlic oil or extract or a synthetic onion or (preferably) garlic essence. The resulting mixture can be stored for a long time without any precautions having to be taken and without any risk of the good anti-mycotic and anti-bacterial effect being lost.

The following Examples are now given, though by way of illustration only, to show details of particularly preferred processes and compositions of the invention.

Example 1

Cinnamon Oil	5 ml
Garlic Oil	5 ml
Ethyl Alcohol	1000 ml

Example 2

Cinnamic Aldehyde	2 ml
Garlic Essence containing essentially diallyl sulfide as flavoring substance dissolved in ethyl alcohol	1 ml
Ethyl Alcohol	1000 ml

Example 3

Cinnamon Oil	3 ml
Onion (A. cepa) oil	3 ml
Ethyl Alcohol	1000 ml

Example 4

Cinnamic Aldehyde	2 ml
Onion essence, containing essentially allyl-propyl sulfide as a flavoring substance dissolved in ethanol	2 ml
Ethyl Alcohol	1000 ml

The cinnamon oil employed in Examples 1 and 3 was cassia oil containing approximately 80% by weight of cinnamic aldehyde. The garlic oil and onion oil employed was ordinary oil produced by the steam distillation of garlic and onion, respectively. The garlic and onion essences employed in Examples 2 and 4, respectively, had a content of active substance of approximately 0.001 %by weight.

Subsequent to having been stored for 13 months, the compositions stated in the Examples still showed no change as regards antimycotic and antibacterial activity. The compositions may be supplied to a foodstuff-product to be treated in any desired manner, e.g. in the form of a spray in a carrier gas such as carbon dioxide, by immersion or brushing. By way of example, the compositions, when employed in practice in connection with the preparation of liver paste, displayed a highly effective antimycotic activity by the surfaces of the liver pastes used in the tests, which were disposed in aluminium trays, being brushed with them. Subsequent to the brushing operation lids were put onto the moulds and the pastes were stored in a refrigerator at 4—6°C. After 20 days these pastes were still completely free of mould fungi although they were taken out of the refrigerator every day and the lid was removed with a view to photographing the contents. Corresponding untreated pastes showed already after 7 days some colonies of mould fungi and, after 14 days had passed, were completely overgrown with these.

Laboratory tests, in which liquid cinnamic aldehyde-product and liquid garlic-product, employed separately, were compared with mixtures of these products, yielded the following results.

The products used for the tests were as detailed below.

A. Cinnamic aldehyde 99—100% by weight. Purchased 17th October, 1970, and the container opened on 22nd September, 1971, in connection with some tests. On 25th March, 1974, a 0.1% by weight solution in ethyl alcohol was produced from the cinnamic aldehyde from this batch with a view to conducting the tests dealt with here.

B. Garlic essence (synthetic). Consisting of 0.001% by weight of an allyl sulfide derivative consisting substantially of diallyl disulfide in ethyl alcohol solution.

C. Garlic extract. Produced by extraction of fresh garlic with ethyl alcohol in the ratio of 10 g garlic to 1000 ml of ethyl alcohol.

D. Mixture of equal parts of (A) (the 0.1% solution) and (B).

E. Mixture of equal parts of (A) (the 0.1% solution) and (C).

F. Mixture of equal parts of a 0.1% by weight solution of cinnamic aldehyde (A) in ethyl alcohol and (B), said mixture being stored in a container from 22nd September, 1971.

The testing technique employed was as described below.

The tests were conducted in conformity with the "disc method" with paper filter roundels having a diameter of 12 mm by the determination of the horizontal degree of diffusion of the tested flavor-products on malt extract-peptone agar which was poured in 9.5 cm Petri dishes. Prior to the tests, each of the dishes with malt extract-peptone agar was brushed with 0.1 ml of a suspension of microbes, which suspension was subsequently allowed to dry up for about 5 minutes. This suspension of microbes was obtained by disposing a drop of distilled water on the root tip of a carrot (purchased from a retailer and taken from a plastic bag containing 400 g carrots). From this drop, which always contains a wide variety of microorganisms of the afore-mentioned kind, three platinum loops of the suspension were transferred to 10 ml physiological salt solution, and the suspension obtained thereby was used to inoculate the dishes. On each of the inoculated dishes, at equal distance from each other, 3 pieces of paper filter roundels of the afore-mentioned type were placed, of which two were impregnated with one of the products A—F while the third was impregnated with only ethyl alcohol as the reference substance. The impregnation was effected by immersing the roundels in the different liquids until saturation and were allowed to dry for 60 seconds before they were placed on the malt extract-peptone agar substrate. The substrate dishes were subsequently incubated for 48 hours at +28°C. In each test 4 dishes were used for determining the degree of diffusion.

The reading of the degree of diffusion of the various products A—F employed for the tests was carried out by radial measurement of the corona surrounding the roundel which, by visual inspection, was free from colonies. In each test the average was calculated of the

8 roundels. The results can be stated as follows.

Designation	Product	Degree of diffusion in mm
A.	Cinnamic aldehyde	<1
B.	Garlic essence	<1
C.	Garlic extract	<1
D.	Cinnamic aldehyde + Garlic essence	<2
E.	Cinnamic aldehyde + Garlic extract	<2
F.	Cinnamic aldehyde + Garlic essence	<3

To the results it has to be observed that the results for both D and E are greater than could be expected from the results of A, B and C. The result of F shows that the effect of the cinnamic aldehyde is retained better when it is fresh at the moment of adding the garlic product than when such addition takes place at a later date when the cinnamic aldehyde has been stored over an extended period after having been exposed to the action of the atmosphere.

WHAT I CLAIM IS:—

1. A process for the protection of foodstuffs (as hereinbefore described) liable to attack by micro-organisms against the attack of such micro-organisms, in which the foodstuffs or a source of infection of the foodstuffs by micro-organisms is treated with an effective amount of cinnamic aldehyde and at least one organic sulphur compound selected from the group consisting of diallyl sulphide, diallyl disulphide, allylpropyl sulphide, allicin and alliin.

2. A process as claimed in Claim 1, in which the cinnamic aldehyde is provided wholly or partially by treating the foodstuff with a cinnamon oil.

3. A process as claimed in Claim 1 or 2, in which the organic sulphur compound is provided wholly or partially by treating the foodstuff with an essential oil from onion or/and garlic.

4. A process as claimed in any of the preceding claims in which the organic sulphur compound is provided wholly or partially by treating the foodstuff with an artificial onion or/and garlic essence.

5. A process as claimed in any of the preceding claims, in which the proportion of cinnamic aldehyde to sulphur compound is from 20:1 to 100:1 by weight.

6. A process for the protection of foodstuffs liable to attack by micro-organisms, the process being substantially as herein described, with reference to the Examples.

7. A composition for use in the process as claimed in any of the preceding claims, the composition comprising cinnamic aldehyde mixed with at least one organic sulphur compound selected from the group consisting of diallyl sulphide, diallyl disulphide, allyl-propyl sulphide, allicin and alliin.

8. A composition as claimed in Claim 7, dissolved, emulsified or suspended in a solvent which is inert with respect to the cinnamic aldehyde and the organic sulphur compound.

9. A composition as claimed in Claim 8, in which the solvent is ethanol.

10. A composition as claimed in any of Claims 7 to 9, in which the proportion of said aldehyde to said sulphur compound is from 20:1 to 100:1 by weight.

11. A composition as claimed in any of Claims 7 to 10, containing a cinnamon oil as the source of said cinnamic aldehyde.

12. A composition as claimed in any of Claims 7 to 11, containing an essential oil from onion and/or garlic as a source of said organic sulphur compound.

13. A composition as claimed in any of the Claims 7 to 12, containing an artificial onion and/or garlic essence as a source of said organic sulphur compound.

14. A composition as claimed in Claim 7 and substantially as herein described, with reference to the Examples.

CRUIKSHANK & FAIRWEATHER,
Chartered Patent Agents,
19 Royal Exchange Square,
Glasgow, G1 3AE,
Scotland.
Agents for the Applicants.

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